this discussion of the amendments, this section will address the Examiner's comments by referring to the paragraph numbers used in the Office Action of March 3, 1992.

The informalities noted by the Examiner in paragraph 15 have been corrected.

Claims 7 and 11 were rejected by the Examiner in paragraph 17 under 35 U.S.C. § 101. These rejections should respectfully be withdrawn. The double-stranded hybrids claimed in claims 7 and 11 have patentable utility.

One example of the utility of the double-stranded hybrids is disclosed in the specification. The specification discloses the use of the double-stranded hybrids in a hybridization protection assay which allows Streptococcus pyogenes to be distinguished from its known and presumably most closely related taxonomic or phylogenetic neighbors. The assay consists of labelling an oligonucleotide probe that is complementary to rRNA sequences in Streptococcus pyogenes with a chemiluminescent molelcule, an acridinium ester; the labelled probe is then incubated with target rRNA sequences to form stable hybrid molecules, such as those claimed in claims 7 and 11. These stable hybrid molecules are crucial to detection of Streptococcus pyogenes in such an assay. Labelled double-stranded hybrid molecules are distinguished from labelled single-stranded probe molecules by treating the solution with alkaline

borate which hydrolyzes the single-stranded probe. The double-stranded hybrid remains intact after hydrolysis and can be detected by chemiluminescence; therefore the amount of chemiluminescence remaining after hydrolysis is proportional to the amount of hybrid formed and indicates the amount of Streptococcus pyogenes present in the sample. The hybrid molecules are therefore critical to the invention, the detection of Streptococcus pyogenes.

Because of their importance to the detection of Streptococcus pyogenes in the chemiluminescence hybrdization protection assay; the hybrid molecules claimed in claims 7 and 11 satisfy the requirement of utility under 35 U.S.C. § 101. Applicant respectfully requests that the rejection of claims 7 and 11 be withdrawn.

With regard to the errors noted by the Examiner in paragraphs 18A, 18B, 18C, and 18D, the specification has been corrected. As requested, a Declaration of Sherrol McDonough accompanies this Amendment to support the correction of the typographical error on page 14, line 5, noted in paragraph 18D.

With regard to paragraph 18E, the headings on Table I have been clarified. The spelling of Streptococcus equi has been corrected. Streptococcus "equi" is atypical and biochemically distinct from Streptococcus equi. The two organisms are listed separately by the Center for Disease Control.

With regard to paragraph 18F, the headings on Table II have been clarified to show that the data indicated are for the three probe mix. This is supported by the statement on page 18, lines 11-12, indicating that "the probes do not react with other bacterial species." The 3 blank spaces in Table II have been corrected to indicate that these species were not tested. As this does not add new matter, Applicant has not submitted the declaration noted in paragraph 18G.

With regard to paragraph 18H, the specification has been extensively amended to indicate that the U.S. patent applications incorporated by reference on page 12, line 26,; page 14, line 18; and page 17, line 1, are assigned to Gen-Probe Incorporated. The presently pending application was assigned to Gen-Probe Products Company on August 9, 1991, Reel/Frame 5838/381 and will be assigned to Gen-Probe Incorporated in the near future. Therefore the referenced applications and the presently pending application will be commonly assigned and meet the Examiner's objections under M.P.E.P. 608.01(p)(B).

Claims 1-14 were rejected by the Examiner in paragraph 19 because of the objections to the specification under 35 U.S.C. § 112, first paragraph. The specification has been amended to satisfy 35 U.S.C. § 112 and Applicant respectfully requests that the rejection of claims 1-14 be withdrawn.

With regard to paragraph 20, the Examiner correctly noted that Applicant does not disclose a hybridization assay that uses antibodies. The claims have been amended to indicate that the probes that hybridize to the target nucleic acid sequences consist of nucleic acid and not antibodies. Applicant respectfully requests that the Examiner reverse the rejection of claim 1, which is now cancelled and replaced with claim 15.

With regard to paragraph 21, claims 1-5 were rejected by the Examiner under 35 U.S.C. § 112, first paragraph. This rejection should respectfully be withdrawn. One of the reasons that these claims were rejected was because of the cross-hybridization of probe 2 or 3 with Streptococcus "equi" indicated in Table II. The claims have been amended, and new claims added, to indicate that Applicant has claimed two types of probes: one that can distinguish Streptococcus pyogenes from all other species of Streptococcus, and one that can distinguish Streptococcus pyogenes and Streptococcus "equi" from all other species of Streptococcus.

Applicant has not only enabled the selection of the three oligonucleotide probes described as an example, but has enabled the selection and synthesis of other probes with sequences not specifically described in the disclosure. One of ordinary skill in the art of hybridization probe design would be able to construct an appropriate probe without undue

experimentation by following applicant's disclosure. Applicant's disclosure teaches the selection of probe sequences with the desired selectivity and hybridization strength. While the Examiner is correct that a single base change could change the specificity of an oligonucleotide probe, Applicant has fully described a method of selecting probe sequences specific to Streptococcus pyogenes. Once these sequences are selected, oligonucleotides can be sequenced and screened for specificity using a simple experimental procedure. Pages 7-11 of the specification provide guidelines for the selection of specific probe sequences. These guidelines detail the importance of selecting a GC-rich sequence to increase the stability of the probe under stringent conditions, the need to minimize the length of perfect complementarity to non-target organisms, as well as the importance of positioning the probe to span as many destabilizing mismatches to non-target organisms as possible. The preferred length of the oligonucleotide probes is also disclosed. In addition Applicant explains the need to avoid probe sequences that are self-complementary or that form strong internal secondary structures. By using the commercially available computer programs described, and by following the Applicant's detailed guidelines, the selection and synthesis of a Streptococcus pyogenes-specific probe is enabled by the

specification. Claims 1-5 are therefore fully enabled by the disclosure and their rejection should respectfully be withdrawn.

With regard to paragraph 22A, claims 1-5 have been amended to meet the Examiner's objections. Claim 1 has been cancelled, corrected, and added as new claim 15.

With regard to paragraph 22B, claim 1 has been cancelled and rewritten as new claim 15 to meet the Examiner's objection.

With regard to paragraph 22C, claim 4 has been cancelled and rewritten as new claim 17 to meet the Examiner's objection.

With regard to the Examiner's objections to claim 6, in paragraphs 22D, 22E, and 22F, claim 6 has been cancelled and rewritten as new claim 19. The specification has been amended to delete the references to corresponding regions of E. coli rRNA.

With regard to paragraph 22G, claims 7 and 11 have been amended to meet the Examiner's objections.

With regard to paragraphs 22H and 22I, claim 11 has been amended to meet the Examiner's objections.

With regard to paragraphs 22J and 22K, claims 12 and 13 have been amended to meet the Examiner's objections.

With regard to paragraph 22L, claim 14 has been amended to meet the Examiner's objection.

In paragraph 23, claim 4 was rejected under 35 U.S.C. § 112, fourth paragraph. Applicant has cancelled this claim and the claim has been rewritten as new claim 17 to meet the Examiner's objections.

Claims 19-20 are added as new claims based on their support in the specification. Claim 19, and dependent claims 20-22, refer to an oligonucleotide that is stable at 60°C in a lithium succinate buffered solution containing lithium lauryl sulfate. These conditions are stated on page 12, line 29 to page 13, line 1 of this patent application. These conditions are specifically disclosed in the Arnold et al. Patent Application Serial No. 613,603, incorporated by reference on page 12, lines 26-28. Example 7, page 31, lines 1-18 of the Arnold et al. application, details a hybridization assay using chemiluminescence to measure the amount of hybridized probe. This assay is performed under the conditions stated in claim 17. The probe length described in claim 20 is supported in the specification at page 4, line 30. The probe length described in claim 21 is supported in the specification at page 8, line 28. The probe length described in claim 22 is supported in the specification at page 4, line 31.

Claims 1, 2 and 5 were rejected by the Examiner in paragraph 25 under 35 U.S.C. § 102(b) as being anticipated by Kilpper-Bälz and Schleifer (FEMS Microbiology Letters 24, 355-365

(1984)). Kilpper-Bälz and Schleifer distinguished Streptococcus pyogenes from some other Streptococcus species by using whole labelled 23S RNA as a probe. The paper does not disclose the use of oligonucleotide probes to distinguish between the streptococci, where oligonucleotides are nucleotide polymers that are shorter than the whole 23S rRNA. Accordingly, claim 1 has been amended and rewritten as new claim 15 to indicate that the claimed probes are oligonucleotides. Applicant believes that claim 1, and dependent claims 2 and 5 are now allowable and respectfully requests that the Examiner withdraw their rejection.

With regard to paragraph 26, claims 1-5 were rejected by the Examiner under 35 U.S.C. § 102(b) as being anticipated by Ludwig et al. (J. Gen. Microbiol. 131, 543-551 (1985)). Ludwig lists sequences of oligonucleotides isolated from the 16S rRNA of various species of Streptococcus and Enterococcus. Some of the sequences were found by Ludwig to be specific to Streptococcus pyogenes as compared to the other eleven strains of bacteria analyzed. The Examiner admits that Ludwig does not refer to the oligonucleotides as probes nor are these sequences used as probes. The oligonucleoides are only subject to a sequence comparison.

The Examiner points to the longest oligonucleotide, a 15-mer, and claims that it might be used as a probe. This is not the case, since the sequence to which it would hybridize is

identical in S. pyogenes and Group C streptococcus. There is a distinct difference between an oligonucleotide that has a sequence specific to Streptococcus pyogenes and a probe that only hybridizes to Streptococcus pyogenes and does not cross-hybridize to other closely related species. While it is possible that this 15-mer sequence might hybridize to Streptococcus pyogenes 16S rRNA, it is not at all clear that it would be able to distinguish Streptococcus pyogenes from other species of streptococcus. As disclosed by the Applicant, a sequence comparison is only the first step in designing a probe that can distinguish Streptococcus pyogenes. The sequences must next be analyzed according to the guidelines disclosed in the specification. The sequence should avoid long A and Trich sequences, the ends should be terminated with G:C base pairs, the probes should be positioned to avoid complementarity to non-target organisms, and the sequence should not form strong internal structures inhibitory to hybridization. Ludwig has only identified a few sequences that are specific to Streptococcus pyogenes as compared to the other 11 bacterial strains. The article did not analyze these sequences under the guidelines Applicant has disclosed; the Ludwig article does not teach how the listed sequences can be used to distinguish Streptococcus pyogenes from other species of Streptococcus.

Furthermore, even if one of the sequences disclosed by Ludwig is specific to Streptococcus pyogenes there is nothing in the Ludwig article which suggests that an oligonucleotide of the same sequence could distinguish Streptococcus pyogenes and not cross-hybridize with other species of Streptococcus. It would not have been obvious to one in the art at the time the invention was made that a probe designed to recognize Streptococcus pyogenes would not cross-hybridize with other bacteria.

Specifically, it is not at all clear that the sequences disclosed by Ludwig could distinguish Streptococcus pyogenes from other closely related species. Applicant has disclosed a method of selecting sequences that can distinguish Streptococcus pyogenes from at least 17 other species of Streptococcus (Table I, pages 17-18) and at least 64 other closely related bacteria (Table II, pages 18-19). By comparison, Ludwig compared sequences derived from Streptococcus pyogenes with only 9 species of Streptococcus (Table 1, pages 545-547). This limited amount of data cannot indicate whether the oligonucleotide sequences disclosed by Ludwig could distinguish Streptococcus pyogenes from enough other species of Streptococcus to be valuable as a diagnostic assay.

As noted above, a computer search by Applicant indicates that the Ludwig 15-mer is not capable of

distinguishing Streptococcus pyogenes from all other streptococci.

Therefore, the Ludwig article clearly does not anticipate Applicant's invention and the rejection of claims 1-5 should respectfully be withdrawn.

In paragraph 27, the Examiner rejected claim 1 under 35 U.S.C. § 102(b) as being anticipated by Scott and Fischetti (U.S. Patent No. 4,784,948 (Nov. 15, 1988)). Scott discloses a probe derived from the M protein gene of Streptococcus pyogenes. As the Examiner admits, the probe disclosed by Scott hybridizes to several species of Streptococcus. Scott does not claim a probe capable of distinguishing Streptococcus pyogenes from other species of Streptococcus; the probe to the M protein gene would be incapable of distinguishing Streptococcus pyogenes because it hybridizes to many other species of Streptococcus. Nothing in the Scott reference suggests that one could construct a probe that is capable of distinguishing Streptococcus pyogenes from other species of Streptococcus. Claim 1 has been rewritten as new claim 15 to reduce the ambiguity in the term "other" and to indicate the particular species from which Applicant's oligonucleotide probe can distingish Streptococcus pyogenes. As Scott does not disclose a probe with this property, the Scott patent does not anticipate Applicant's invention. Therefore,

Applicant respectfully requests that the Examiner's rejection of claim 1 be withdrawn.

With regard to paragraph 28, claims 12-14 have been rewritten and are no longer dependent on claims 8-10. Therefore claim 11 no longer intervenes between claims 8-10 and claims 12-14.

With regard to paragraph 29, claim 12 has been amended to meet the Examiner's objection. Applicant appreciates the Examiner's evaluation of claim 11 on the merits.

With regard to paragraph 30, Applicant appreciates the notice that claims 6-14 are allowable over the prior art.

Accordingly, the claims are now allowable and a notice to that effect is respectfully requested. If there is any fee in connection with this response, please charge Deposit Account 12-2475 for the appropriate amount.

Respectfully submitted,

LYON & LYON

Dated: July 6, 1992 By _____
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